Toxicity of Insecticides in a Glass-Vial Bioassay to Adult Brown, Green, and Southern Green Stink Bugs (Heteroptera: Pentatomidae)

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ABSTRACT Adult brown, *Euschistus servus* (Say); green, *Acrosternum hilare* (Say); and southern green, *Nezara viridula* (L.), stink bugs were collected from soybean, *Glycine max* (L.) Merr., in fall 2001 and 2002 near Stoneville, MS, and Eudora, AR. A glass-vial bioassay was used to determine LC_{50} values for the three species of stink bugs for the pyrethroids bifenthrin, cypermethrin, cyfluthrin, λ-cyhalothrin, and permethrin, and the organophosphates acephate, dicrotophos, malathion, and methyl parathion. Results confirmed findings of other researchers that the brown stink bug was less susceptible to pyrethroid and organophosphate insecticides than were green and southern green stink bugs. The susceptibility of all three stink bug species to the insecticides tested was very similar at both test locations. The study established baseline insecticide mortality data from two locations in the mid-South for three stink bug species that are pests of soybean and cotton, *Gossypium* spp. Data from the tests are valuable for future use in studies on resistance and in resistance monitoring programs.

KEY WORDS brown stink bug, green stink bug, southern green stink bug, insecticides

SEVERAL STINK BUG SPECIES are found in cotton in the United States (Emfinger et al. 2001). The most abundant and important species include green stink bug, *Acrosternum hilare* (Say); southern green stink bug, *Nezara viridula* (L.); and brown stink bug, *Euschistus servus* (Say) (Turnipseed 1973, Roach 1988). The use of transgenic cotton to control lepidopterous pests and boll weevil, *Anthonomous grandis grandis* Boheman, eradication have resulted in reduced insecticide use in cotton, *Gossypium* spp., in the United States. This reduction has allowed numbers of stink bugs found in cotton to increase, with higher numbers more frequently found in transgenic cotton (Turnipseed et al. 1995, Bachelor and Mott 1996, Greene and Turnipseed 1996). Because the majority of the cotton now grown in the mid-South is transgenic, stink bugs have become an increasingly important pest in cotton. In 1995, 95,311 ha of cotton was estimated to have been infested with stink bugs in Arkansas, Louisiana, Mississippi, and Tennessee, of which 2,570 ha was treated for stink bugs (Williams 1996). The corresponding numbers in 2002 were 835,393 ha infested with 352,208 ha treated (Williams 2003).

Stink bugs can cause significant damage to cotton and reduce yield and fiber quality (Wene and Sheets 1964, Roach 1988, Barbour et al. 1990, Turnipseed et al. 1995, Turnipseed and Greene 1996, Greene et al. 1998). Stink bugs are controlled in cotton with insecticides. The recommended insecticides for stink bug control in cotton in Mississippi include the organophosphates acephate, dicrotophos, and methyl parathion, and the pyrethroids cyfluthrin, cyhalothrin, deltamethrin, and tralomethrin (Layton 2002). Pyrethroids are recognized as being more effective for green and southern green stink bugs compared with brown stink bugs (Emfinger et al. 2001, Layton 2002, Willrich et al. 2003).

Information on current insecticide susceptibility levels in stink bugs is needed to detect changes in susceptibility that could occur in stink bug populations over time and at different locations. Glass-vial bioassays are one rapid method for measuring insecticide resistance in an insect population. Emfinger et al. (2001) used a glass-vial bioassay to show that the pyrethroid bifenthrin was equally toxic to brown and southern green stink bugs, whereas cypermethrin, cyfluthrin, and λ-cyhalothrin were significantly more toxic to southern green than they were to brown stink bugs. They also found that bifenthrin was more toxic to brown stink bugs compared with southern green stink bugs by exposing adults in the laboratory to cotton bolls treated in the field. Willrich et al. (2003) established baseline data (LC_{50} values) for brown and southern green stink bugs from Franklin Parish, LA, by using a glass-vial bioassay and the organophosphates acephate and dicrotophos, and the pyrethroids bifenthrin, cypermethrin, cyfluthrin, and λ-cyhalothrin. Both of the studies (Emfinger et al. 2001, Willrich et al. 2003) used a glass-vial bioassay modified from the methods used by Plapp et al. (1987) and

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Table 1. Results from testing adult green (G) and southern green (SG) stink bugs from Eudora, AR, and Stoneville, MS, with a glass-vial bioassay to determine LC_{50} values for pyrethroid insecticides

Insecticide	Location	Species	*n*	Slope ± SE	LC_{50}	95% CL	χ^2	$P > \chi^2$
Permethrin	Stoneville	G	180	1.17 ± 0.17	2.03	1.5–2.9	2.40	0.49
		SG	210	0.92 ± 0.11	2.81	2.1–3.8	2.64	0.62
	Eudora	G	180	1.07 ± 0.20	0.89	0.7–1.0	2.96	0.40
		SG	210	0.98 ± 0.12	4.46	3.5–5.6	7.75	0.10
Bifenthrin	Stoneville	G	150	1.04 ± 0.17	0.32	0.2–0.4	1.85	0.40
		SG	180	0.75 ± 0.11	0.48	0.3–0.7	4.05	0.26
	Eudora	SG	150	1.11 ± 0.28	0.82	0.5–1.2	1.07	0.58
Cyfluthrin	Stoneville	G	150	0.42 ± 0.11	0.19	0.1–0.4	1.15	0.56
		SG	180	0.67 ± 0.10	0.21	0.1–0.3	4.55	0.21
	Eudora	SG	180	0.92 ± 0.12	0.24	0.2–0.3	2.84	0.42
Cypermethrin	Stoneville	G	210	0.59 ± 0.08	0.22	0.1–0.3	6.09	0.19
		SG	180	0.70 ± 0.11	0.30	0.2–0.4	2.50	0.47
	Eudora	SG	180	0.64 ± 0.10	0.47	0.3–0.7	6.89	0.08
λ-Cyhalothrin	Stoneville	G	210	0.79 ± 0.10	0.21	0.2–0.3	3.45	0.49
	Eudora	SG	180	0.89 ± 0.13	0.12	0.1–0.2	2.40	0.49

LC_{50} values are in micrograms per vial.

Snodgrass (1996) in which the exposure period for stink bugs was 4 h. The current study was conducted to determine insecticide susceptibility (LC_{50} values) to five pyrethroid and four organophosphate insecticides in brown, green, and southern green stink bug populations in the Mississippi River Delta. The study included testing of stink bug populations at two different locations in Arkansas and Mississippi.

Materials and Methods

Adult stink bugs were collected for testing with a sweep net from soybean, *Glycine max* (L.), located near Stoneville, MS, and Eudora, AR, during September and October 2001 and 2002. Stoneville is located in Washington County, whereas Eudora is located in Chicot County along U.S. Highway 65, ≈64 km (40 miles) south of Stoneville. Although collections were made over a 2-yr period, the abundance of the three stink bug species varied each year. Consequently, in some cases all insecticides used in the bioassays were not tested against all three stink bug species at each location. Adults were separated by species in the laboratory and held in paper ice cream cartons (3.8 liter) for a 24-h period to allow any injured bugs to die before testing. Adults were examined as they were used in the tests and parasitized adults were discarded. Green bean pods, *Phaseolus vulgaris* L., were provided as a food source for adults in the containers. The beans were washed in detergent and soaked in a 3% sodium hypochlorite solution as described in Snodgrass (1996) to remove or oxidize any insecticide residue on them. The containers were stored under laboratory conditions at 24–26 °C, and humidity was not controlled.

A glass-vial bioassay (Snodgrass 1996) developed to test insecticide resistance in *Lygus lineolaris* (Palisot de Beauvois) was modified and used to determine the amount of insecticide resistance present in each stink bug population tested. In this bioassay, adult stink bugs were placed into 20-ml glass scintillation vials that had been treated with a test insecticide. The insecticide was applied by pipetting 0.5 ml of acetone with the insecticide into each vial, which was then rolled on its side until an even layer of insecticide dried on its inner surface. Vials were rolled on a hotdog cooker (Star MFG, Smithville, TN) whose heating element was disconnected. Control vials received only the 0.5 ml of acetone, and in all tests the insecticide was applied to the vials on the same day the test was performed. Changes from the bioassay described in Snodgrass (1996) were that no food (green bean pod) was provided in the vials and that only one adult stink bug was tested per vial. Preliminary testing showed that unlike *L. lineolaris*, all three species of stink bugs had high survival without food or water for time periods up to 48 h in the glass vials.

Vials were stored upright in the laboratory and mortality was determined after 24 h. Adults were considered dead if they were unable to right themselves or walk, or there was no movement when they were prodded with a metal probe. All bioassays had three to four replications of five to eight different concentrations of the insecticide being tested. Each replication consisted of 10 vials with one adult stink bug per vial. Technical grade insecticides were purchased from Chem Service (West Chester, PA). The insecticides tested included the pyrethroids bifenthrin, permethrin, λ-cyhalothrin, cyfluthrin, and cypermethrin, and the organophosphates acephate, dicrotophos, malathion, and methyl parathion. Mortality for treated vials was corrected for natural mortality in the nontreated vials by using Abbott's formula (Abbott 1925). Corrected data from bioassays were analyzed with the PROC PROBIT option of SAS (SAS Institute 1997).

Results

LC_{50} values for green and southern green stink bugs tested with the five pyrethroids were all <1 μg per vial, with the exception of permethrin (Table 1). Green stink bugs were slightly more susceptible than southern green stink bugs at both locations when tested with permethrin, bifenthrin, cyfluthrin, and cypermethrin. Malathion was the least toxic organophosphate tested with green and southern green stink

Table 2. Results from testing adult green (G) and southern green (SG) stink bugs from Eudora, AR, and Stoneville, MS, with a glass-vial bioassay to determine LC_{50} values for organophosphate insecticides

Insecticide	Location	Species	*n*	Slope ± SE	LC_{50}	95% CL	χ^2	$P > \chi^2$
Malathion	Stoneville	G	180	1.60 ± 0.25	26.40	22.5–30.5	0.28	0.96
		SG	210	1.00 ± 0.15	19.50	15.8–24.2	2.75	0.60
	Eudora	SG	210	0.95 ± 0.21	17.67	2.5–24.3	1.62	0.81
Dicrotophos	Stoneville	G	150	1.09 ± 0.18	2.09	1.5–2.7	0.43	0.81
		SG	180	1.00 ± 0.14	1.23	0.9–1.6	3.21	0.36
	Eudora	SG	150	1.05 ± 0.19	1.83	1.3–2.6	3.36	0.34
Acephate	Stoneville	G	180	0.96 ± 0.17	7.50	5.9–9.6	3.40	0.33
		SG	210	1.20 ± 0.17	5.41	4.3–6.5	6.81	0.15
	Eudora	G	210	0.92 ± 0.12	9.38	7.4–12.1	3.00	0.56
		SG	150	0.93 ± 0.22	7.98	5.6–11.0	3.89	0.42
Methyl parathion	Stoneville	G	210	0.67 ± 0.08	0.54	0.4–0.8	6.03	0.20
		SG	180	0.72 ± 0.10	0.26	0.2–0.4	1.88	0.60
	Eudora	G	180	0.78 ± 0.12	1.30	1.0–1.8	4.08	0.25
		SG	180	0.94 ± 0.14	0.72	0.5–0.9	5.80	0.12

LC_{50} values are in micrograms per vial.

bugs, with LC_{50} values as much as 100-fold higher than the other organophosphates tested (Table 2). LC_{50} values for methyl parathion ranged from 0.26 to 1.30 μg per vial for green and southern green stink bugs at both locations, and methyl parathion was the most toxic organophosphate tested for both species. Dicrotophos also was very toxic to green and southern green stink bugs, with LC_{50} values ranging from 1.23 to 2.09 μg per vial. Acephate was more toxic than malathion to green and southern green stink bugs with LC_{50} values at both locations ranging from 5.41 to 9.38 μg per vial.

Brown stink bugs were more tolerant to pyrethroid insecticides than were green or southern green stink bugs. Bifenthrin and cyfluthrin were close in their toxicity to brown stink bugs, with LC_{50} values ranging from 0.72 to 1.28 μg per vial at both locations (Table 3). Permethrin was the least toxic pyrethroid to brown stink bugs with LC_{50} values 3- to 13-fold higher than were found with the other pyrethroids. Toxicity to brown stink bugs found with cypermethrin was between that found with permethrin and bifenthrin or cyfluthrin with LC_{50} values of 3.06 (Stoneville) and 3.12 (Eudora) μg per vial. Malathion was the least toxic organophosphate tested with brown stink bugs and had LC_{50} values at both locations as much as 29-fold higher than the other organophosphates tested (Table 3). Methyl parathion and dicrotophos had very similar toxicity to brown stink bugs with LC_{50} values ranging from 2.33 to 3.11 μg per vial. The toxicity of acephate to brown stink bugs was between that of dicrotophos or methyl parathion and malathion with LC_{50} values of 7.77 (Stoneville) and 10.94 (Eudora) μg per vial.

Discussion

The LC_{50} values found with bifenthrin for brown and southern green stink bugs from Eudora and Stoneville were similar and ranged from 0.48 to 1.28 μg per vial (Tables 1 and 3). This indicated that bifenthrin was about equally toxic to adults of both species. Emfinger et al. (2001) and Willrich et al. (2003) found bifenthrin to be equally effective against brown and

Table 3. Results from testing adult brown stink bugs from Eudora, AR, and Stoneville, MS, with a glass-vial bioassay to determine LC_{50} values for pyrethroid and organophosphate insecticides

Insecticide	Location	*n*	Slope ± SE	LC_{50}	95% CL	χ^2	$P > \chi^2$
Pyrethroid							
Permethrin	Stoneville	210	0.92 ± 0.14	9.28	7.3–11.6	5.21	0.27
	Eudora	210	0.95 ± 0.12	8.87	6.5–11.3	2.03	0.73
Bifenthrin	Stoneville	150	1.34 ± 0.19	1.28	1.0–1.6	2.91	0.23
	Eudora	150	0.95 ± 0.10	0.72	0.5–1.1	2.40	0.49
Cyfluthrin	Stoneville	150	0.65 ± 0.14	0.93	0.5–1.6	2.01	0.57
	Eudora	150	0.70 ± 0.15	0.76	0.4–1.2	5.40	0.15
Cypermethrin	Stoneville	210	1.19 ± 0.16	3.06	2.5–3.7	7.85	0.10
	Eudora	210	1.38 ± 0.17	3.12	2.6–3.7	7.50	0.11
Organophosphate							
Malathion	Stoneville	240	1.04 ± 0.14	67.13	55.5–80.8	2.47	0.78
	Eudora	210	0.54 ± 0.11	50.87	34.9–75.7	2.31	0.68
Dicrotophos	Stoneville	150	1.29 ± 0.20	3.11	2.5–3.8	0.04	0.98
	Eudora	150	1.02 ± 0.19	2.33	1.6–3.4	3.81	0.28
Acephate	Stoneville	150	1.06 ± 0.18	7.77	6.1–10.1	2.62	0.27
	Eudora	210	1.35 ± 0.16	10.94	9.0–13.1	3.19	0.53
Methyl parathion	Stoneville	150	1.44 ± 0.26	3.02	2.3–3.8	1.67	0.80
	Eudora	180	1.01 ± 0.15	2.40	1.8–3.0	1.80	0.61

LC_{50} values are in micrograms of insecticide per vial.

southern green stink bug adults and nymphs in glass-vial bioassays and field tests. Bifenthrin and three other pyrethroids were tested using topical application against brown and southern green stink bug adults and nymphs (Greene et al. 2001). All of the pyrethroids were effective against southern green stink bug adults and nymphs, and bifenthrin was the most effective pyrethroid for adults and nymphs of the brown stink bug. The efficacy of bifenthrin for both brown and southern green stink bugs is important because both species frequently occur together in cotton, and whereas most recommended pyrethroids control southern green stink bugs, they are less effective than bifenthrin for brown stink bugs.

Willrich et al. (2003) used a glass-vial bioassay to show that the southern green stink bug was significantly more sensitive than brown stink bugs to the pyrethroids cyfluthrin, cypermethrin, and λ-cyholathrin. Results from the current study showed that the brown stink bug had 2- to 10-fold higher LC_{50} values for cypermethrin, cyfluthrin, and permethrin than the southern green stink bug (Tables 1 and 3). Green stink bugs were found to have LC_{50} values similar to those found for southern green stink bugs for bifenthrin, cypermethrin, and cyfluthrin. This is important in the mid-South where both green and southern green stink bugs can be found in cotton.

Results from field tests in soybean and cotton have indicated that organophosphate insecticides recommended for stink bug control are still effective (McPherson et al. 1999, Willrich et al. 2000, Fitzpatrick et al. 2001). The LC_{50} values found for brown stink bugs were two- to four-fold higher for malathion and 3- to 12-fold higher for methyl parathion than the LC_{50} values found with the two insecticides with green and southern green stink bugs at both locations (Tables 2 and 3). However, the LC_{50} values for dicrotophos for brown, green, and southern green stink bugs from Eudora and Stoneville were similar ranging from 1.23 to 3.11 μg per vial. This indicated that dicrotophos was equally toxic to all three stink bug species at the two locations. Willrich et al. (2003) found that southern green and brown stink bugs did not differ significantly in their susceptibility to dicrotophos. They also found acephate to be 6.4- to 7.4-fold more toxic to brown stink bugs than was dicrotophos. Our findings were the opposite of this, dicrotophos was 2.5- to 4.7-fold more toxic to brown stink bugs than was acephate (Table 3). The reason(s) for this difference in the results of the two studies is unknown. The glass-vial bioassay we used to determine insecticide resistance in the stink bugs was identical to the glass-vial bioassay used by Willrich et al. (2003), except they used a 4-h exposure period, whereas our exposure period was 24 h. Our results from the glass-vial bioassay were similar to the results they obtained in their glass-vial bioassay. For the pyrethroids tested in both studies (bifenthrin, cypermethrin, and cyfluthrin), they obtained LC_{50} values ranging from 0.05 to 0.58 and from 0.27 to 1.69 μg per vial with southern green and brown stink bugs, respectively. Our LC_{50} values for these pyrethroids were higher and ranged from 0.21 to 0.82 μg per vial for southern green stink bugs and from 0.72 to 3.12 μg per vial for brown stink bugs. The longer exposure period we used may have produced the higher LC_{50} values we obtained, but with the exception of acephate, the results of the two studies showed the same trends with respect to the toxicities of the insecticides to the stink bugs.

The susceptibility of populations of the three species of stink bugs to various insecticides needs to be determined at many other locations throughout the mid-South before the overall status of insecticide tolerance in these pests can be determined. This also could lead to detection of populations with higher tolerance levels, which could be studied to determine the cause(s). Information of this type will aid the development of resistance management programs.

In summary, the study provided additional baseline data on current tolerance levels to pyrethroid and organophosphate insecticides in brown, green, and southern green stink bugs at two separate locations. The study confirmed the findings of other researchers that brown stink bugs were less susceptible to pyrethroid and organophosphate insecticides than were green or southern green stink bugs. The susceptibility of all three stink bug species to the insecticides tested was very similar at both test locations.

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